

The undersigned hereby states that the written Sequence Listing submitted concurrently herewith does not include matter which goes beyond the content of the application as filed and that the information recorded on the diskette submitted concurrently herewith is identical to the written Sequence Listing.

Applicants believe that no fee is required for the submission of this Sequence Listing, since it is being submitted within the one-month deadline from the mailing date of the Notice to Comply. However, if a fee is required, the Commissioner is authorized to deduct such fee from the undersigned's Deposit Account No. 50-0990. Please deduct any additional fees from, or credit any overpayment to, the above-noted Deposit Account

December 23, 2002

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APPENDIX A  
MARKED UP PARAGRAPHS ILLUSTRATING AMENDMENTS MADE TO  
SPECIFICATION

Inserted text is indicated by double underlining

In the paragraph located on page 14, line 26:

Figure 6 shows the nucleic acid sequence corresponding to subtilisin E (SEQ ID NO: 4).

In the paragraph located on page 35, lines 18-27:

In an alternative embodiment of the methods of assembling synthetic and mutagenized gene libraries that are mediated by single-stranded templates, described above, oligonucleotides are synthesized in such a way as to end in a single redundant codon. For example, this is accomplished by first preparing two batches of resin containing either \*N-N-G—resin or \*N-N-C-resin (where \* indicates the attachment end at which new bases are added during synthesis). This can be accomplished using an automated DNA synthesizer according to methods known in the art. For example, a fixed mass (e.g., 10 mg) of \*N-N-C is added to the reaction vessel following each trinucleotide coupling set. All subsequent reaction steps are then shared by the progressively accumulated resin. Fresh resin is added after each trinucleotide synthesis step to allow generation of an oligo with a redundancy at each position. As shown in Figure 7A, invariant recombination and digestion sites are optionally incorporated within the backbone structure derived from the oligonucleotide sequences (identified herein as SEQ ID NOs: 5-16). As an alternative to the single base coupling cycle described above, vials containing preformed trinucleotides encoding the amino acid or set of amino acids desired at a given position are optionally included. As shown in Figure 7A, the transfer # indicates the trinucleotide synthesis step at which the progenitor resin is added in order to give the listed sequence. For example, each transfer is optionally transferred to a single synthesis vessel in which the same base is added to each oligonucleotide at each reaction cycle after the redundant codon is incorporated.

In the paragraph located on page 144, lines 4-12:

Transfer of the library to the pBE shuttle vector, followed by transformation into *B. subtilis* and selection of antibiotic resistant transformants by growth on nutrient-antibiotic plates allows for secretory expression and immediate and direct, on-plate measurement of activity and thermostability screening as reported by Zhou et al. (1998), *supra*, using the succinyl-ala-ala-pro-phe-p-nitroanilide (SEQ ID NO: 1) (s-AAPF-pNa) method of Zhou and Arnold (1997), *supra*. This assay allows for rapid assessment of the thermostability of the clones derived from the template-based recombination process.

In the paragraph located on page 146, lines 24-30:

a. Oligonucleotide primers PBADGFP3 (P-ATAAGATTAGCGGATCCTAC) (SEQ ID NO: 2) and PBADGFP4 (P-TCGGGCATGGCACTCTTGAA) (SEC ID NO: 3) - which flank the random stop sites in pBAD(Cm)GFP(c3)STOP1 (e.g., 'STOP1 phagemid') - were phosphorylated and used to prime amplification of corresponding 500 base pair fragments from the STOP1 and STOP2 phagemids using the TthXL thermostable polymerase mix according to manufacturer's protocol.